

Appl. No. : **09/582,817**
Filed : **November 8, 2000**

SUMMARY OF INTERVIEW

Exhibits and/or Demonstrations

None

Identification of Claims Discussed

30, 31, 34, 40, 41, 44, 45, 47, and 64

Identification of Prior Art Discussed

None

Proposed Amendments

In claim 30 to specify that microchannels are not grooves.

Principal Arguments and Other Matters

Additionally, the shapes of the CD's were discussed.

Results of Interview

Applicant agreed to amend the claims whereby grooves will be distinguished from microchannels.

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REMARKS

Applicant wishes to thank Examiner Sisson for the courtesy extended to Daniel Altman, attorney of record, and Marina Gordey, agent of record, on March 21, 2005. The present response to the outstanding Office Action includes the substance of the Examiner Interview.

Disposition of Claims

Claim 30 has been amended to clarify that grooves are not microchannels. Support for the amendment can be found in the Specification as filed, for example, page 7, lines 28-31; page 18, lines 27-30; and page 20, lines 1-3. Support for the additional amendments can be found in Claims 44 and 47, which have been canceled without prejudice. Claim 41 has been amended to clarify the meaning of "the signal". Support for this amendment can be found in Claim 30. Claim 45 has been amended to correct dependency. Therefore, no new matter has been introduced by these amendments.

Compliance with 35 USC §112, first paragraph

The Examiner has rejected Claims 30, 31, 34, 40, 41, 44, 45, 47 and 64 under 35 USC §112, first paragraph, as not compliant with the written description requirement. Applicant respectfully disagrees. The rule under MPEP 2163 is that:

"To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention.

Possession may be shown in a variety of ways including description of an actual reduction to practice, or by showing that the invention was "ready for patenting" such as by the disclosure of drawings or structural chemical formulas that show that the invention was complete, or by describing distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention.

The description need only describe in detail that which is new or not conventional. What is conventional or well known to one of ordinary skill in the art need not be disclosed in detail. See *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d at 1384, 231 USPQ at 94. If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate description requirement is met.

Patents and printed publications in the art should be relied upon to determine whether an art is mature and what the level of knowledge and skill is in the art. In most technologies which are mature, and wherein the knowledge and level of skill in the art is high, a written description question should not be raised for original claims even if the specification discloses only a method of making the

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invention and the function of the invention. See, e.g., *In re Hayes Microcomputer Products, Inc. Patent Litigation*, 982 F.2d 1527, 1534-35, 25 USPQ2d 1241, 1246 (Fed. Cir. 1992) ("One skilled in the art would know how to program a microprocessor to perform the necessary steps described in the specification. Thus, an inventor is not required to describe every detail of his invention. An applicant's disclosure obligation varies according to the art to which the invention pertains. Disclosing a microprocessor capable of performing certain functions is sufficient to satisfy the requirement of section 112, first paragraph, when one skilled in the relevant art would understand what is intended and know how to carry it out.").

In ¶ 6, the Examiner interpreted Claim 30 as not using any means to protect or otherwise shield the registered data from the binding of nucleic acids or the generation of any precipitate thereon. Applicant wishes to point out that, as recited in the claim, the binding of the target and capture molecules occurs in areas separated from the areas comprising registered data and that, as recited in the claim as amended herein, unbound target molecules are removed.

In ¶ 7, the Examiner has interpreted Claim 30 as encompassing the reading of registered information prior to, during or after having conducted the binding and detecting parts of the assay, and wherein the surface of the CD and or DVD has not been treated as to remove any materials bound to its surface. Applicant wishes to point that Claim 30 specifies that the binding assays occur while the disc is not rotating, while the reading of the registered data is performed when the disk is rotating. Furthermore, Claim 30 has been amended to now recite the step of removing unbound target molecules after the binding between the target and the capture molecules on the non-rotating disk has occurred.

In ¶ 8 the Examiner has interpreted Claim 30 as encompassing there being no means to identify just where on the surface the capture molecules are located, or means by which the reader head can accurately and reproducibly locate, read and record the signal. Claim 30 has been amended to additionally recite that the registered data is binary data which comprises characteristics and position of capture molecules fixed upon specific areas of said CD or DVD or interpretation of the signal resulting from the binding between the target and the capture molecules. Accordingly, reading of the binary data allows the apparatus to determine where on the disc binding occurred and to identify the capture molecule at that position.

In ¶ 9 the Examiner has interpreted Claim 30 as encompassing the conditions whereby non-specific binding occurs. Claim 30 has been amended to now recite the step of removing

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unbound target molecules after the binding between the target and the capture molecules on the non-rotating disk has occurred.

In ¶ 10-12 the Examiner has interpreted Examples 1-5 as not individually describing the full invention as presented in Claim 30. The rule is that the written description can be satisfied by describing distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention. Here, each of the examples focuses individually on each of such distinguishing identifying characteristics of the invention and, together, the Examples provide all the information necessary to describe the claimed invention. Example 1 provides binding between said target molecule and a capture molecule fixed upon a side of the surface of a solid support, said solid support consisting of a compact disc (CD) or digital video disc (DVD), treating said CD or DVD in order to obtain a detectable signal resulting from the binding of the target molecule and said capture molecule, wherein said binding results in an opaque precipitate on said CD or DVD, wherein said CD or DVD is not rotating on its axis; removing unbound target molecules; Examples 2 and 3 provide detecting a signal, and reading the registered data, and reading the signal resulting from the binding between said target molecule and said capture molecule; Figures 4, 5 and 7 depict an apparatus comprising two different reading devices.

In ¶ 12, the Examiner asserts that none of the Examples teach using a DVD. Applicant wishes to point that the Specification on page 5 teaches that the definition of a disk includes a CD or a DVD which comprise data that can be read by a CD-reading device. Furthermore, the rule is that the description need only describe in detail that which is new or not conventional. What is conventional or well known to one of ordinary skill in the art need not be disclosed in detail. Here, the CD and DVD technology was already quite mature at the time the invention was made (December 30, 1997), as is apparent from the attached "History of CD Technology" (Exhibit 1) and as described in The CD-ROM Handbook, 2nd edition (referenced in the Specification as filed, page 13, lines 29-31). Therefore, the inventor did not have to describe in detail as to how to use a DVD instead of a CD as a person skilled in the art would recognize that a DVD can be used instead of a CD in the method of Claim 30.

In ¶ 13 the Examiner states that while the specification sets forth but a single embodiment of the method of detection of a DNA on the CD, it does not give sufficient detail as to how to detect more than one molecule of the capture molecule or sufficient detail of an assay where

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reagents are allowed to bind to one another while disc is spinning. Applicant respectfully disagrees. The rule is that:

If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate description requirement is met.

Here, the Specification provides enough description of the binding assays which allow production of a signal which can be detected by one of the two reading devices: see page 22, line 3 through page 24, line 30, and examples 1 and 3 (streptavidin-peroxidase assay), example 2 and 4 (silver staining). These assays are well-known to person skilled in the art as standard methods of visualizing binding, and therefore their detailed description is not required.

Furthermore, Claim 30 specifically recites that the binding is performed when the disc is not rotating.

In ¶ 14 the Examiner states that the specification is silent as to how one is to read binary registration data when the surface of the CD has been coated with an agent that would limit non-specific binding and the coating of the entire surface of the CD would effectively block the reading of the binary data. Applicant respectfully disagrees. Applicants maintain that this limitation is not recited in the claims, and therefore it is not necessary to provide the written description for it. In addition, the Specification describes in several places that such protective layer does not impede the laser reading of the registered data (see, page 9, line 31 through page 10, line 3, as well as Example 3).

In ¶ 15 the Examiner states that in accordance with Claim 34, detection and/or quantification of the target molecules is done by detecting variations of a magnetic field, however the Example 5 allegedly does not provide in sufficient detail as to how capture molecules are positioned on non-grooved surfaces, means of locating the capture molecules, or means to identify/correlate the signal from the binding of capture and target molecules. Applicant respectfully disagrees. The rule is:

The description need only describe in detail that which is new or not conventional. What is conventional or well known to one of ordinary skill in the art need not be disclosed in detail. See *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d at 1384, 231 USPQ at 94. If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of

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filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate description requirement is met.

Here, the ferromagnetic precipitate is a result of using a commercially available kit according to the instructions therein. It is clear, that the technology for detecting the presence of such precipitate was conventional at the time the invention was made, including the detectors and the programs to analyze the data. Therefore, the Specification needed only to describe in detail that which is new and not conventional, i.e. the CD where such binding, precipitation and detection are envisioned to occur.

In ¶ 16-17 the Examiner states that the Specification does not provide adequate written description of various shapes of a CD. Applicant respectfully disagrees. Here, the Specification provides explicit description that the disc can be in any "external" shape or form as long as it allows the rotation of said disc along the central axis (11:20-25). The Specification provides a representative number of species of the CD forms: circular, elliptic, hexagonal, octagonal, square or triangle. In the response to the previous Office Action, filed December 6, 2004, Applicant provided evidence that CDs and DVDs may be manufactured in a variety of forms. Applicant did not intend this evidence to be a pre-filing art, but instead used it to show how these shapes were described in the Specification as filed, as well as to show that the exact "external" shape of the CD (DVD) is irrelevant to the ability of these discs to be read by a CD/DVD reader.

In ¶ 18-19 the Examiner has construed the tracks of the CD as being microchannels. The Examiner further stated that figure 7 which shows two different reading devices does not show any binding members present and therefore it only shows that a false/uninformative signal will be obtained. Applicant respectfully disagrees. Claim 30 as currently amended specifies that the grooves are not microchannels. The grooves are not coextensive with the areas of the disc where the capture molecules are located. Further, figure 7 shows two different reading devices (one laser for reading registered data in CD tracks, and one magnetic head reader for detecting the presence of a ferro-magnetic compound depicted in the areas of the CD devoid of registered data in CD tracks. The binding members are not shown in this figure, because the purpose of the figure is not such that the capture molecules are pictured in detail. Rather, the figure indicates that in those areas where specific binding occurs a precipitate forms which can be detected by the magnetic head reader (second reader).

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In ¶ 20-21 the Examiner has not accepted attorney's arguments presented in the previous response to Office Action that one skilled in the art would appreciate that the same methodology employed for detection upon a CD can be employed on a DVD as well. As discussed above, the CD and DVD technology was already quite mature at the time the invention was made. Therefore, a person skilled in the art at the time the invention was made would have known that a DVD can be used instead of a CD in the method of the present invention. Therefore the inventor did not have to describe in detail as to how to use a DVD instead of a CD.

In ¶ 22-23 the Examiner has stated that the Specification does not provide an adequate written description as to how the assay to be conducted so as to minimize non-specific binding, nor how the assay is conducted when two or more reading devices all read the same signal, variations in magnetic field. Applicant respectfully disagrees. Examples, of how to minimize non-specific binding is described for example on page 27, lines 21-29, page 30, lines 4-5. As for the two reading devices, Claim 30 specifies that the reading devices are different so that a first one reads the binary registered data, while the second reading device reads the signal resulting from the binding to a portion of the disk which does not contain registered data.

In ¶ 24-27 the Examiner had not accepted the argument provided in the response to the previous Office Action regarding rejection of Claim 47 for the lack of written description of how a signal resulting from the binding between a target and capture molecule is interpreted. Applicant respectfully disagrees. Claim 47 has been canceled, and the subject matter of Claim 47 was incorporated into Claim 30. Support for the registered data on the disc of currently amended Claim 30 which comprises characteristics and position of the capture molecules or interpretation of the signal resulting from the binding of the target molecule to the capture molecule can be found in the Specification as follows:

"compact disc <...> also contains data which allows the disc to be read in a laser-based CD reader (information usually stored as a series of pits localized in the disc grooves and which are necessary to localize the <...> capture molecule on the surface of the disc. This can be obtained through the presence of appropriated [sic!] pits or protruding indentations equivalent to the pits in the disc grooves" (18:27-19:2)

"<...> the optical detection system which can read said binding may comprise a photo-diode which can detect a small light beam and which moves according to a one dimension axe [sic!] so as to cover the radius of the disc <...>. Combined with the rotation of the disc, such focused photo-detection scans the entire surface of the disc <...> to assay for the target molecule present at any location on the disc. The preferred detection device is the photo-diode of the commercially available CD readers which are used for music, video or software CD (see Fig. 5).

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The photo system can be servocontrolled in order to stay in focus to the detection surface. if a second optical detection system is provided for the detection of the signal, it can also be servocontrolled or linked to the other one for its control, or it may receive from the first one data in order to adjust its focus and its tracks on the disc. The data received from the consecutive reading of the disc surface can also be stored in a computer, reformatted if necessary and analyzed for the definition of spots localization.” (21:3-23)

<...> an insoluble product [of binding between the target and the capture molecules] <...> will precipitate <...> and form <...> mounds on the surface of the disc [on] <...> the disc surface that will be illuminated by the (laser) beam. The reflected (laser) beam intensity will be lowered when illuminating the precipitate and a perturbation in the (laser) reflection can be obtained. Such a perturbation is analyzed by the photosensitive detection device as a pit upon the surface of the disc.” (23:15-27)

<...> signal can be read either as a binary signal or as an absolute value. The binary signal <...> processed as an electronic computerized data and analyzed by appropriate software. This software will convert this information into data which can analyze the detection obtained and quantity the binding between the target molecule and its <..> capture molecule. Preferably, the disc <...> comprise additional pits <...> which give information about the type, the quantity and the specificity of <...> capture molecule.” (24:31-25:12)

See also detailed description about how any information is encoded and read on a CD: 5:25-6:4; 9:1-21; 10:6-11:19; 13:13-14:2. Furthermore, if the Examiner is asserting that an actual computer program is required to satisfy a written description requirement, then, as discussed above, the rule is:

In most technologies which are mature, and wherein the knowledge and level of skill in the art is high, a written description question should not be raised for original claims even if the specification discloses only a method of making the invention and the function of the invention. See, e.g., *In re Hayes Microcomputer Products, Inc. Patent Litigation*, 982 F.2d 1527, 1534-35, 25 USPQ2d 1241, 1246 (Fed. Cir. 1992) (“**One skilled in the art would know how to program a microprocessor to perform the necessary steps described in the specification. Thus, an inventor is not required to describe every detail of his invention. An applicant's disclosure obligation varies according to the art to which the invention pertains. Disclosing a microprocessor capable of performing certain functions is sufficient to satisfy the requirement of section 112, first paragraph, when one skilled in the relevant art would understand what is intended and know how to carry it out.**” (emphasis ours))

Therefore, Applicant asserts that Claim 30 as currently amended is supported in the Specification as filed.

In ¶ 30 The Examiner has rejected Claims 30, 31, 34, 40, 41, 44, 45, 47 and 67 under 35 USC §112, second paragraph as being indefinite. More specifically, Claim 30 was asserted to be confusing in that the solid surface is a CD or a DVD, which are known to have grooves on their

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surface (i.e. “microchannels”), while Claim 30 recites that the disc is not having microchannels. Claim 30 has now been amended to clarify that grooves are not microchannels. Support for the amendment can be found in the Specification as filed, for example, 7:28-31; 18:27-30; and 20:1-3. Therefore, Claim 30 is now definite. Claim 41 was rejected for lack of antecedent basis in Claim 30 for reciting “detection and/or quantification of the signal”. Applicant has amended Claims 30 and 41 accordingly. Claim 47 was rejected under 35 USC §112, second paragraph and 35 USC §101 for reciting a use without any active, positive steps delimiting how this use is actually practiced. Claim 47 has been cancelled and the subject matter of Claim 47 is now in Claim 30 which has been amended to recite that the registered data comprises characteristics and localization of capture molecules fixed upon specific areas of said CD or DVD or interpretation of the signal resulting from the binding between the target and the capture molecule. Support for this amendment can be found, for example, on page 19, lines 27-32.

Therefore, Claims 30 and 41 as well as claims 31, 34, 40, 44, 45, and 67 are now definite, and their rejection under 35 USC §112, second paragraph should be withdrawn.

In addition, applicant provides the following proof of the existence of the commercial embodiment of the claimed invention: an on-line advertisement of a BioCD from the Eppendorf Array Technologies website (Exhibit 2); and a publication by Alexandre et al 2002 “Compact Disc with both Numeric and Genomic Information as DNA Microarray Platform” BioTechniques 33:435-439 (Exhibit 3).

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CONCLUSION

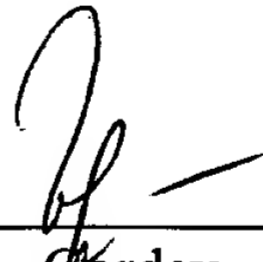
Applicants have endeavored to address all of the Examiner's concerns as expressed in the outstanding Office Action. Accordingly, amendments to the claims, the reasons therefor, and arguments in support of the patentability of the pending claim set are presented above. Any claim amendments which are not specifically discussed in the above remarks are made in order to improve the clarity of claim language, to correct grammatical mistakes or ambiguities, and to otherwise improve the capacity of the claims to particularly and distinctly point out the invention to those of skill in the art. In light of the above amendments and remarks, reconsideration and withdrawal of the outstanding rejections is specifically requested. If the Examiner finds any remaining impediment to the prompt allowance of these claims that could be clarified with a telephone conference, the Examiner is respectfully requested to initiate the same with the undersigned.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: June 15, 2005

By: 
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Registration No. 52,950
Agent of Record
Customer No. 20,995
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042405

EXHIBIT 1



1.800.340.1633

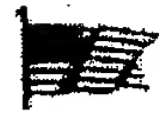
Main Site

CD
Products &
Services

DVD
Products &
Services

Ordering
Process

About
OneOff



History of CD Technology



History of OneOff

Mission Statement

History of CD
Technology



- 1841 Augustin-Louis Cauchy Proposes a Sampling Theorem.
- 1842 Charles Babbage Proposes analytical engine for performing and storing calculations.
- 1854 George Boole publishes "An Investigation Into the Laws of Thought." A book that contained, among other things, theories that were later used to build digital circuits.
- 1855 Leon Scott de Martinville invents the phonoautograph, a machine that records vibrations on a carbonized paper cylinder.
- 1876 Alexander Graham Bell introduces the telephone
- 1877 Thomas Edison invents the phonograph while trying to invent a device that would record and repeat telegraphic signals (digital)
- 1887 Emily Berliner replaces Edison's wax cylinder phonograph with the audio disc.
- 1915 78 R.P.M records introduced
- 1922 J.R. Carson examines the idea of time sampling for communications
- 1928 Harry Nyquist publishes "Certain Topics in Telegraph Transmission Theory." His theory contained proof that the technology used in today's audio cd's could work.
33 1/3 Records Introduced
- 1937 A. Reeves invents pulse code modulation (PCM), a technology used by computers and CD's for audio in the present day.
H. Aiken from Harvard approaches IBM and proposes a electrical computing machine.
- 1943 The U.S. Army turns on the first computer (ENIAC) at the University of Pennsylvania.
- 1947 Magnetic Tape Recorders hit the U.S. market.
- 1948 The transistor is invented by Bell Laboratories.
Claude E. Shannon publishes "A Mathematical Theory of Communication." -- Yet another important development for theories used in CD technology
- 1949 45 rpm records hit the U.S. market, thanks to microgroove technology.
- 1950 Richard W. Hamming publishes information about error detection/correction codes. It would be impossible for CD's to work without error correction.
- 1958 Invention of the Laser.
Stereo LP's produced.
Integrated Circuit introduced by Texas Instruments
- 1960
Computer Music experiments take place at major laboratories.
I.S. Reed and G. Solomon publish information on multiple error correction codes. These come to be known as the "Reed-Solomon"

Codes which are the codes used for encoding and reading CD's.
Working Laser produced.

1967 NHK Technical Research Institute demonstrates a 12-bit PCM digital audio recorder with a 30 kHz (30,000 times per second) sampling rate. The digital recording goes onto a high-grade video tape.

1969 Sony introduces it's 13-bit PCM digital recorder at a 47.25 kHz (47,250 times per second) sampling rate. The digital recording is sent to a 2" video tape.

Klass Compaan, a Dutch physicist comes up with the idea for the Compact Disc.

1970 At Philips, Compaan and Pete Kramer complete a glass disc prototype and determine that a laser will be needed to read the information.

1971 Microprocessor produced by Intel
Digital Delay line used by BBC's studios (first digital audio device).

1972 Compaan and Kramer produce color prototype of this new compact disc technology

1973 BBC and other broadcast companies start installing digital recorders for master recordings.

1977 Mitsubishi, Hitachi & Sony show digital audio disc prototypes at the Tokyo Audio Fair.

JVC Develops Digital Audio Process

1978 Philips releases the video disc player

Sony sells the PCM-1600 and PCM-1 (digital audio processors)

"Digital Audio Disc Convention" Held in Tokyo, Japan with 35 different manufacturers.

Philips proposes that a worldwide standard be set.

Polygram (division of Philips) determined that polycarbonate would be the best material for the CD.

Decision made for data on a CD to start on the inside and spiral towards the outer edge.

Disc diameter originally set at 115mm.

Type of laser selected for CD Players.

1979 Prototype CD System demonstrated in Europe and Japan.

Sony agrees to join in collaboration.

Sony & Philips compromise on the standard sampling rate of a CD -- 44.1 kHz (44,100 samples per second)

Philips accepts Sony's proposal for 16-bit audio.

Reed-Solomon code adopted after Sony's suggestion.

Maximum playing time decided to be slightly more than 74 minutes.

Disc diameter changed to 120mm to allow for 74 minutes of 16-bit stereo sound with a sample rate of 44.1 kHz

1980 Compact Disc standard proposed by Philips & Sony.

1981 Matsushita accepts Compact Disc Standard

Digital Audio Disc Committee also accepts Compact Disc Standard.

Sharp achieves production of semiconductor laser.

Philips & Sony collaboration ends.

1982 Sony & Philips both have product ready to go.

Compact Disc Technology is introduced to Europe and Japan in the fall.

1983 Compact Disc Technology is introduced in the United States in the

- spring
The Compact Disc Group formed to help market.
CD-ROM Prototypes shown to public
30,000 Players sold in the U.S.
800,000 CD's sold in the U.S.
- 1984** Second Generation & Car CD players introduced.
First Mass Replication Plant in the United States built.
Portable (i.e., Sony DiscMan) CD Players sold.
- 1985** Third generation CD Players released.
CD-ROM drives hit the computer market.
- 1986** CD-I (Interactive CD) concept created.
3 Million Players sold in U.S.
53 Million CD's sold in U.S.
- 1987** Video CD format created.
Allen Adkins of Optical Media International joins with SonoPress in
Amsterdam and demonstrates a desktop system for pre-mastering CD's
(Adkins and SonoPress, produced a replicated CD in less than 24-hours
using this system).
- 1988** CD-Recordable Disc/Recorder Technology Introduced
- 1990** 28% of all U.S. households have CD's.
9.2 million players sold annually in the United States.
288 million CD's sold annually in the United States.
World Sales close to 1 Billion
- 1991** CD-I format achieved.
CD-Recordable Introduced to the Market
"QuickTopix" the first CD-R pre-mastering Software introduced by
Allen Adkins.
- 1992** CD-R Sales reach 200,000
- 1996** DVD Technology Introduced.
Prices of Recorders and CD-R Media go down significantly.
High Demands cause World-Wide CD-R Media Shortage.
- 1997** DVD Released.
DVD Players/Movies hit consumer market.
DVD-R standard created (3.9 Gig).
Mitsui builds it's first CD-R production plant in the U.S.
World-wide shortage ends.
Price of CD-R media lower than ever imagined.
- 1998** DVD-RAM, DVD-Recordable systems/equipment hits market.
DVD-Video/ROM authoring tools hits the market.
CD-R prices continue to drop.
- 1999** DVD-Video Becomes main stream.
Consumers begin purchasing DVD Players & Movies on a mass level.
Most major film studios have titles on DVD.
DIVX Dies (DIgital Video eXpress).
Second Generation DVD Burners.
4.7 Gig DVD-R Media Developed.

Source

1841-1991

1991-1999

Pohlmann, Ken C.

"The Compact Disc Handbook, 2nd
Edition" (Click to See this Book at
Amazon.Com)

Copyright 1992 & 1989 A-R Editions,
Inc.

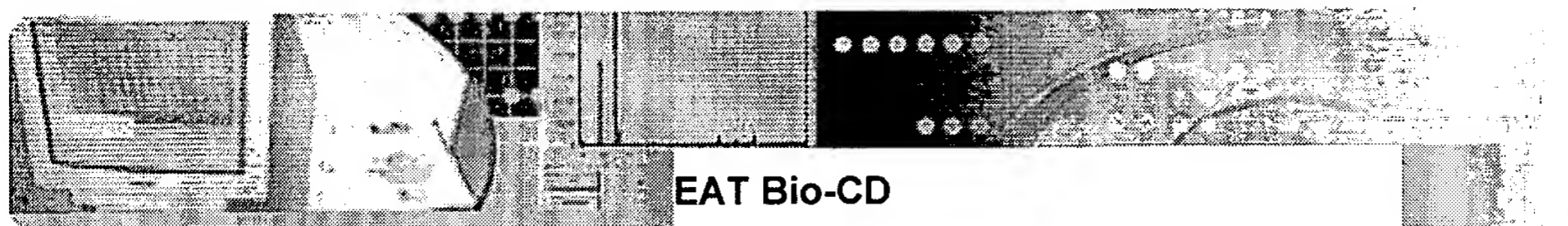
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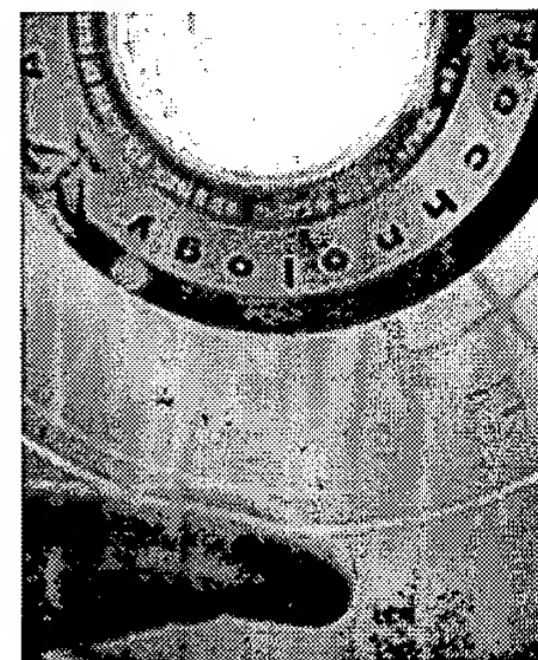
EXHIBIT 2



Combining biological and digital information on the same surface

Main features of the Bio-CD:

- Up to 20 arrays per disc reducing cost per array
- Convenient for both research and diagnostic applications
- Ultra-sensitive colorimetric detection using EAT Silverquant® technology
- Results of analysis stored on Bio-CD recordable track



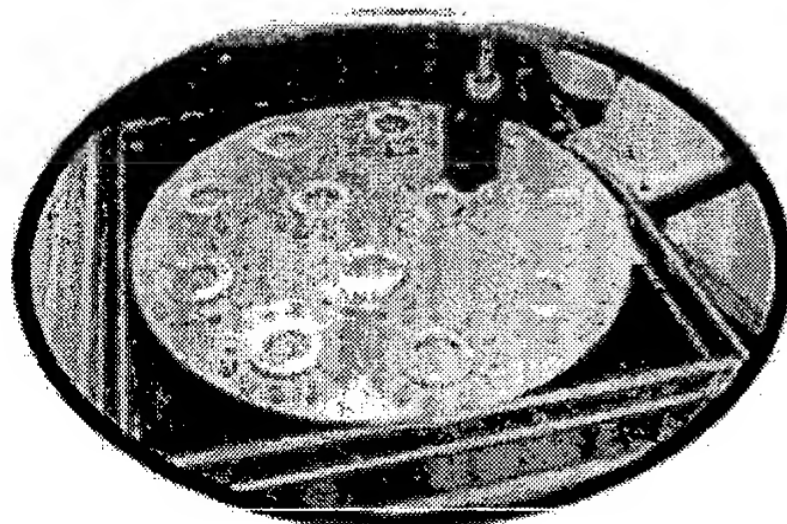
Bio-CD: microarrays on compact disc

Full technology platform has been developed



Close view of arrays on CD

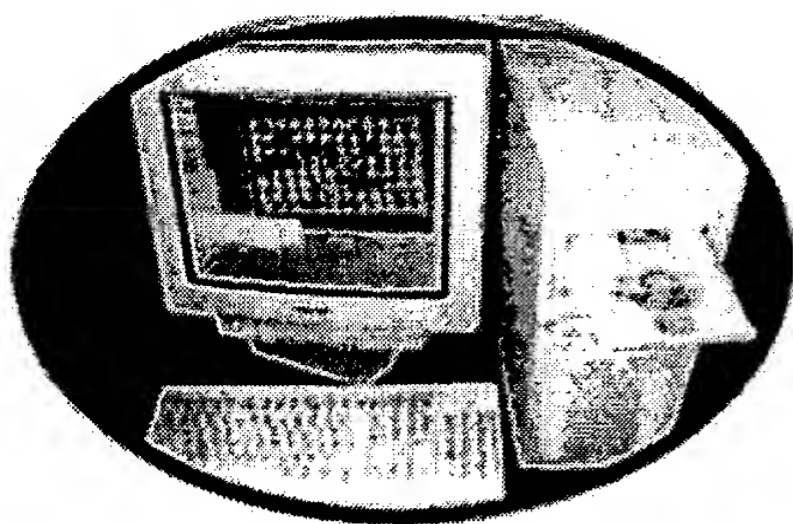
Functionalization of the Bio-CD using proprietary resins



Bio-CD arrayer

Bio-CD arrayer: custom CD-spotter having a capacity of 20 discs

Bio-CD Reader: quantify and burns biological datas on the recordable track of the Bio-CD



Bio-CD reader

Please click [here](#) for an overview of the Bio-CD reader

Related publication: Compact Disc with Both Numeric and Genomic Information as DNA microarray platform, I. Alexandre, Y. Houbion, J. Collet, S. Hamels, J. Demarteau, J-L Gala, BioTechniques 33-435-439, 2002 / 08

This technology and the products are available for licensing in several applicational areas.

Your licensing and OEM contact:

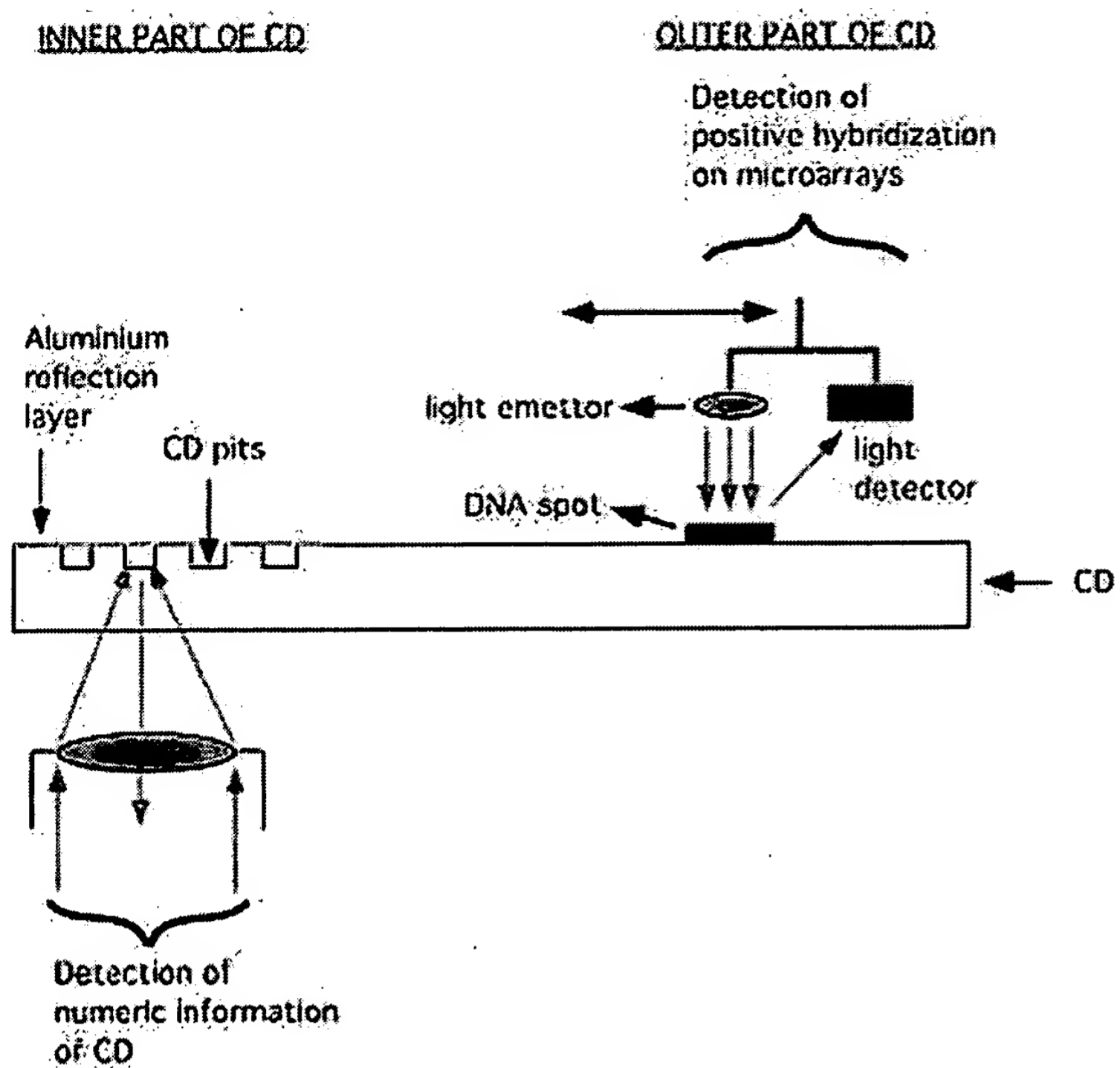
Jürgen Lindemeier, Ph.D.

Eppendorf AG

Barkhausenweg 1

22339 Hamburg

lindemeier.j@eppendorf.de



Design of the Bio-CD reader

AVAILABLE COPY

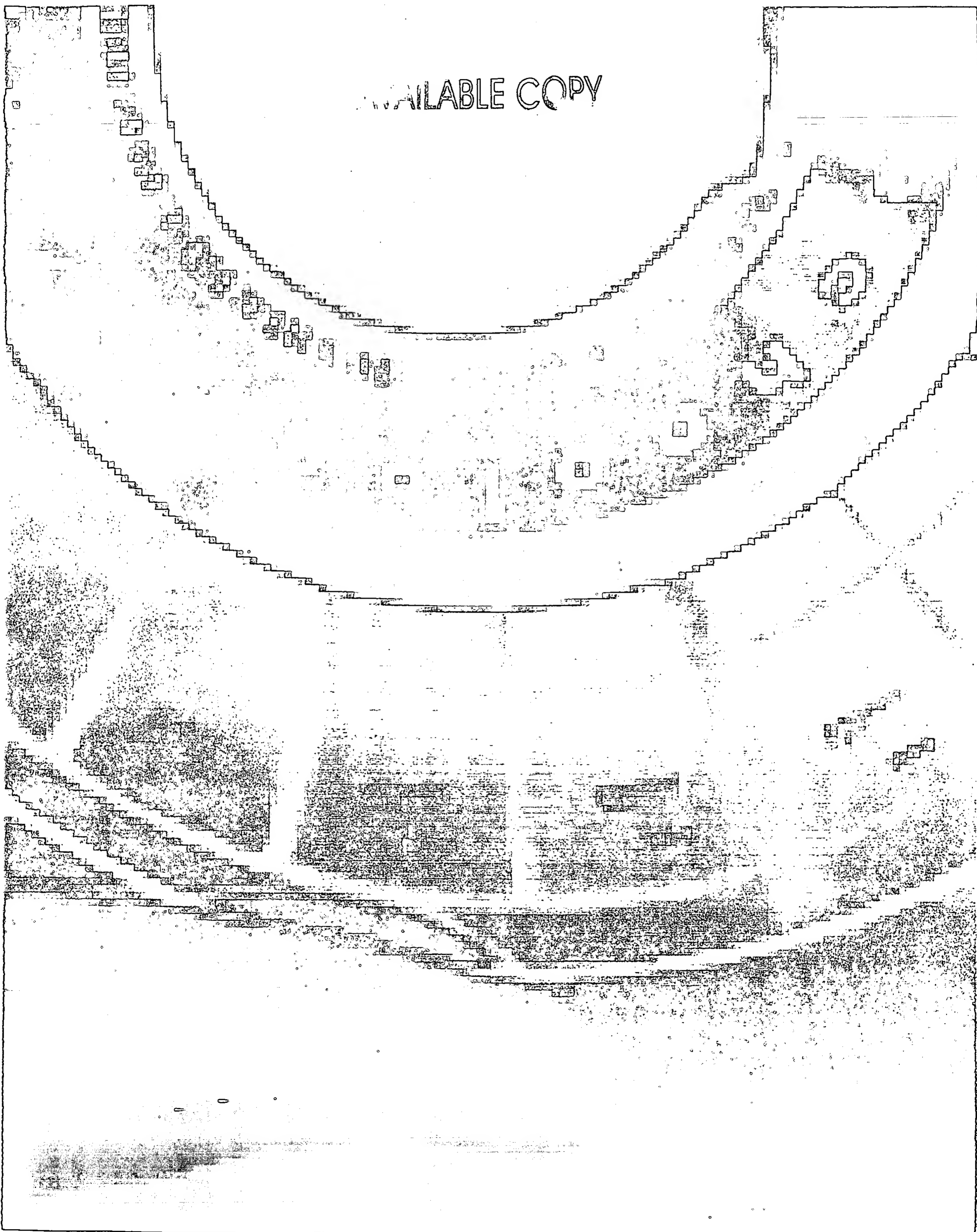


EXHIBIT 3

PRODUCT APPLICATION FOCUS

A forum for manufacturers to describe the current and potential applications of new research instruments or products.

Compact Disc with Both Numeric and Genomic Information as DNA Microarray Platform

I. Alexandre, Y. Houbion, J. Collet¹, S. Hamels, J. Demarteau¹, J.-L. Gala², and J. Remacle

FUNDP and ¹Advanced Array Technology (AAT), Namur, and ²Saint-Luc University Hospital, Brussels, Belgium

BioTechniques 33:435-439 (August 2002)

ABSTRACT

The compact disc (CD) is an ideal tool for reading, writing, and storing numeric information. It was used in this work as a support for constructing DNA microarrays suited for genomic analysis.

The CD was divided into two functional areas: the external ring of the CD was used for multiparametric DNA analysis on arrays, and the inner portion was used for storing numeric information. Because polycarbonate and CD resins autofluoresce, a colorimetric method for DNA microarray detection was used that is well adapted for the fast detection necessary when using a CD reader. A double-sided CD reader was developed for the simultaneous analysis of both array and numeric data. The numeric data are engraved as pits in the CD tracks and result in the succession of 0/1, which results from the modulation of the laser reflection when one reads the edges of the pits. Another diffraction-based laser was placed above the CD for the detection of the DNA targets on the microarrays. Both readers fit easily in a PC tower. Both numeric and genomic information data were simultaneously acquired, and each array was reconstituted, analyzed, and processed for quantification by the appropriate software.

INTRODUCTION

Miniaturization has been a main target of the electronic industry and is now invading molecular biology through the technology of microarrays or biochips (3–6). The principle of DNA microarray analysis was known for many years as reverse dot blot analysis, which was first performed on large nitrocellulose or nylon membranes. Efforts in miniaturization were associated with new supports. The electronic chips were first proposed for DNA binding using electric-based targeting (9). The possibility of in silico synthesis of nucleotide sequences combined with photolithography provided surfaces with several thousand different small oligonucleotide capture

probes (11,12). Glasses were also activated by introducing aldehyde groups for the covalent binding of aminated DNA capture probes. The miniaturization was obtained for this method through the use of robotic precise automatic spotting (13). In all cases, the hybridized DNA is detected by incorporating fluorescent labels either directly during the copy of target sequences to be analyzed or by a second labeling step with fluorescent streptavidin or antibodies (7,9). The search for electronic-based detection methods (10,11) continues, but the proposed solutions are now becoming of practical use (14).

The search for how to use polymers such as polycarbonate for supporting DNA microarrays was hampered mainly because of their autofluorescence. However, a new colorimetric method has been proposed, based on the deposit of silver precipitation at the DNA location (1,10). This new method allows plastic polymers to be tested as support for the DNA arrays. Although the three-log dynamic range of the colorimetric detection method is lower than the four-log range of the Cy3 fluorescence method, the silver precipitation colorimetric method is as sensitive as the Cy3 fluorescent detection of DNA when it is performed on glass slides (1).

Here we used compact disc (CD) as a support for constructing DNA assays. The goal of the project was to use a common platform for storing numeric and genomic data, both being read by one or two reading devices inserted into a PC tower. The first attempt (data not shown) was to take part of the CD technology developed for reading and engraving the numeric information, using the laser reflection process present in a normal CD reader. Indeed, we succeeded in transferring the DNA hybridization site onto the site of numeric information and read it with a normal CD reader, but the solution was impractical because of the constraint of working in a clean atmosphere. We then decided to separate physically on the CD the location of the encoded numeric information from the DNA binding location. This solution gave the best results and is described in this paper.

MATERIALS AND METHODS

Bio-CD

The commercially available CD is a 1.2-mm-thick polycarbonate disc that carries on its upper side one track running from the internal to the external part of the disc. The track is composed of pits that are 1–4 μm long, 0.15 μm deep, and 0.5 μm wide (2). This upper surface is then covered by a reflective aluminium or gold layer that is then protected from oxidation by a varnish layer.

The pits give binary information in a simple way: a laser beam is focused on the surface of the pits and, when the beam is reflected by a flat surface, gives a 0 value. However, when the reflection meets the edge of a pit, it decreases below a threshold value and this deflection is counted as a 1 value. The succession of the 1/0 numeric signals are then converted into data of different kinds like bytes for computers, some of them necessary for the CD reader to recognize the CD, adjust the speed of rotation, and control the position of the head compared to the CD. The reader follows the track as a continuous spiral on the CD with an elaborate servo tracker (part of the CD reader) that corrects for both lateral and vertical variations.

To avoid interference between the gene-based signal and the numeric reading that also provides the track and controls the speed, we separated the two signals laterally on the CD and detected them separately with two laser-reading devices. For the lateral separation, the CDs were engraved with a numeric information band of around 1 cm located on the inner part of the CD and covered with an aluminium layer restricted to this location. The outer part was a transparent polycarbonate band coated with a DNA fixation layer. This specially designed CD for microarray technology is called the Bio-CD (Figure 1).

Bio-CD for *Staphylococcus* Detection

The DNA spotting, PCR amplification, and hybridization were performed as described previously (8). In brief, the *fem A* gene of the various *Staphylococci* are amplified by a consensus set of primers and then detected on CD microarrays that bear the capture probes specific for the *fem A* of the dif-

ferent *Staphylococci* species. Array spotting on the CD was performed on an arrayer with a plate that could support 12 CDs (Figure 2).

The spots were 300 μm in diameter, and the DNA bound to the CD through the use of a specific fixation layer coated on the CD (UCB, Drogenbos, Belgium).

Colorimetric Silver Labeling

After hybridization, the CD is washed four times for 1 min with 10 mM maleate buffer containing 15 mM NaCl and 0.1% Tween[®] 20, pH 7.5. The CD is incubated with a solution of streptavidin-colloidal gold conjugate and then with the Silver Blue solution (AAT, Namur, Belgium) as described earlier (1). The results are digitized and quantified with software that is included in the workstation (AAT).

Bio-CD Reader

A commercially available CD reader (Creative Laboratories, Singapore, Singapore) was used to read the numeric information written onto the CD. A second laser-based reader was attached on the upper part of the CD reader. The reader consists of a laser diode module that illuminates a 50- μm spot on the surface of the CD with a wavelength of 670 nm (Figure 3). The diffracted light is detected by a photodiode, and the data are digitized by an acquisition card (National Instruments, Austin, TX, USA).

The head of the detector moves following a stepping motor-driven radial displacement with a speed of 20 mm/min while the CD is turning. The overall CD surface devoted to the DNA analysis is a 15-mm-wide external band. This surface is scanned in less than 1 min, and the overall digitized data represent 6 MB of information. The data from each array are retreated to recreate the picture of each array present on the CD. Each array is stored in a separate file. Image analysis is then processed by the evaluation of the average gray level of the pixels of each spot, minus the average gray level of pixels surrounding the spot. The means of quadruplates are then calculated \pm SD.

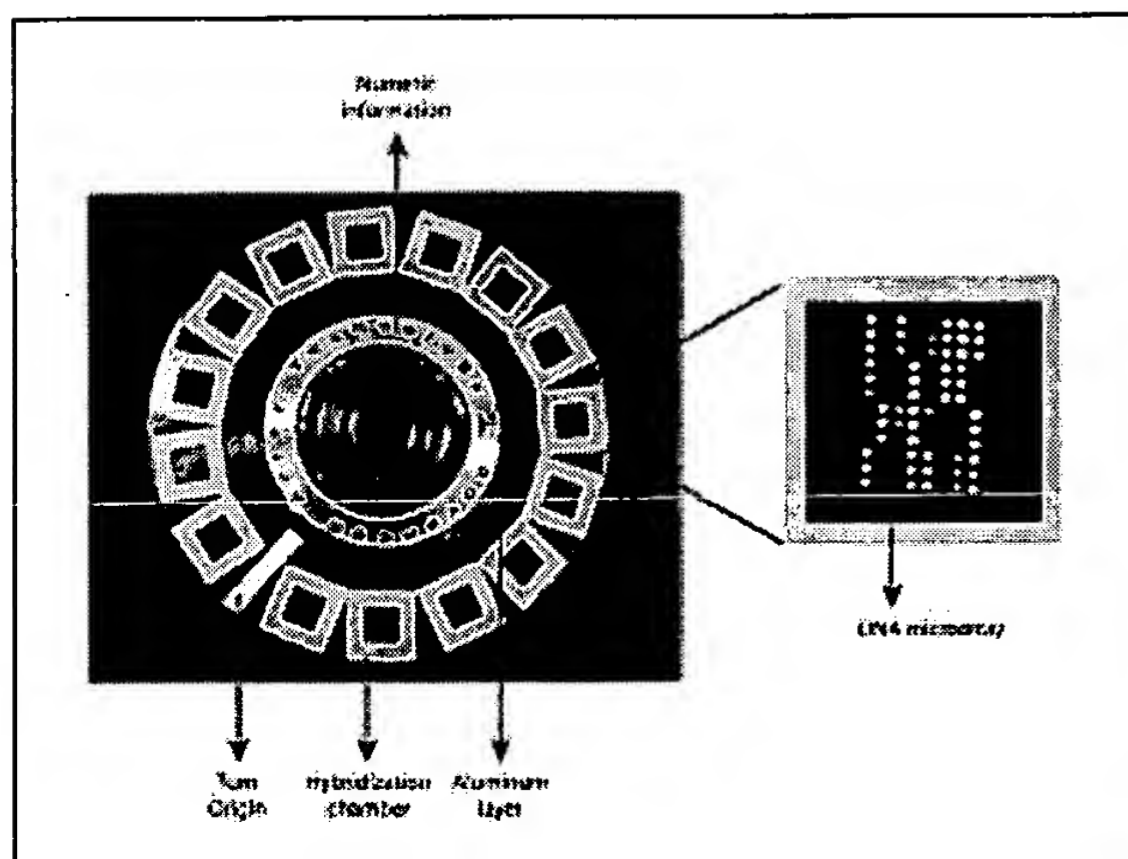


Figure 1. View of a CD used for microarray detection. The center contains numeric information covered by an aluminium layer. The outer part of the CD is covered by 15 hybridization chambers in which a microarray has been spotted.

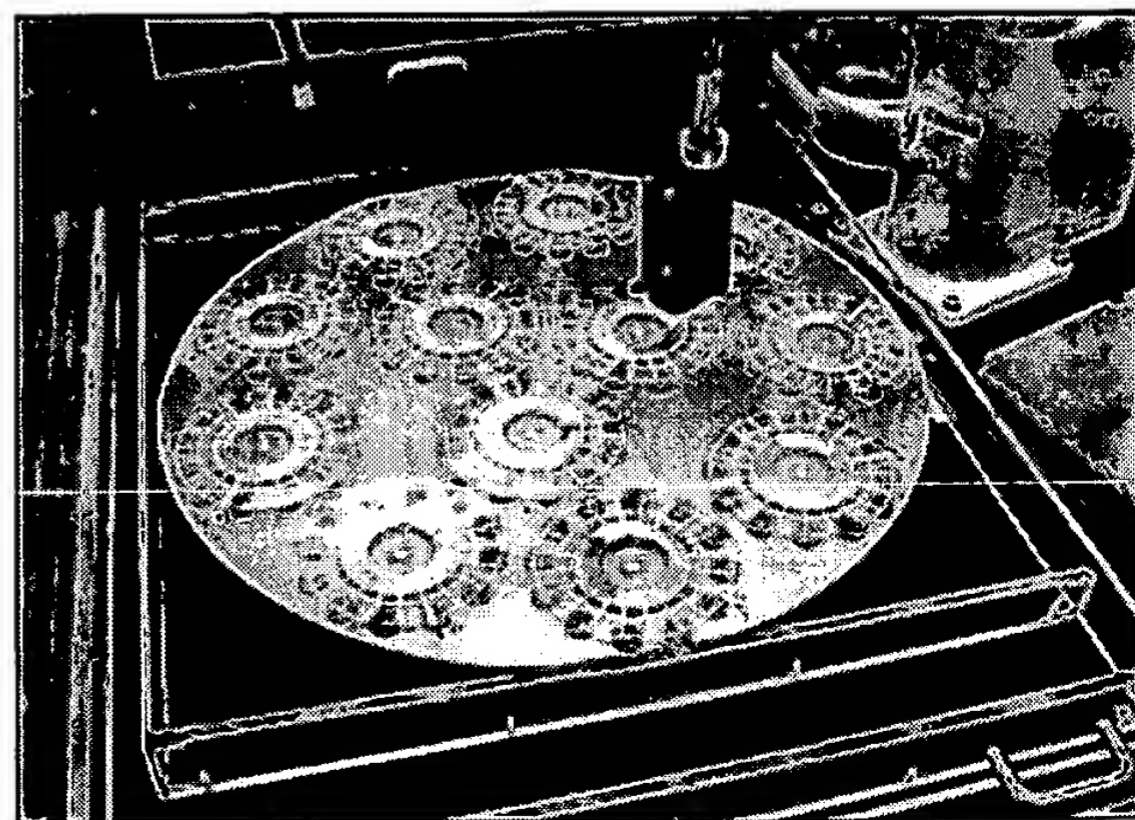


Figure 2. Axial arrayer developed for the transfer of capture probes present in solution from the multi-well plate onto the surface of the CD. The arm of the robot is a θ -based movement, and the disc is fixed on a rotating platform that has 12 CDs.

RESULTS

The optimization of the conditions for silver precipitation and reading with the double CD reader were first performed on biotinylated probes fixed on the CD. Once the detection was optimized, the CD platform was tested using the DNA hybridization assay.

A normal CD reader was used to read the numeric information on the inner part of the CD. This commercial CD reader also controlled the CD rotation speed during the analysis. The analysis of the outer part of the CD carrying the genomic results was performed by a second laser-based reader (Figure 3), which scans the outer part of the CD and measures the scattered light caused by silver precipitates that result from positive hybridization.

The signal was digitized by a PC acquisition card and stored as files, each one corresponding to one array. The data acquisition was then followed by the use of specific data analysis and data mining software.

The CD detection method was first tested for the detection of *Staphylococci* strains as already developed on glass (8). The principle of the method is to amplify a part of the *fem A* gene that is present in all *Staphylococcus* species using consensus primers and to detect them by hybridization on capture probes specific for each of the species. This array detects the five most common *Staphylococci* species. The array also contained a consensus capture probe for the genus *Staphylococcus* identification and a capture probe for the *mec A* gene. The *mec A* gene is associated with the methicillin resistance of the *Staphylococcus*. The image of each array is reconstituted, and the spot analysis was processed as followed. The program first identifies the spots and corrects for the deformation of the rectangular shape of the array given the circular reading on the CD. The average intensity of each spot inside its boundary is then calculated. The mean value of the background around

each spot is subtracted from the spot values. The means of the replicates are then calculated with two standard deviations, and values are assigned to the sequence of a specific bacteria.

A first comparative assay for the detection of the *fem A* and *mec A* sequence from methicillin-resistant *S. epidermidis* on glass slide and on CD is presented in Figure 4. The positive signals are in dark on the glass, resulting from light absorption of the illuminated glass, while they appear as bright signal on the CD because of the laser diffraction detected by the photodiode. Besides this difference, both patterns of hybridization were similar with the *S. epidermidis*, the consensus, and the *mec A* spots being positive on both arrays.

The experiment was then extended with a single CD spotted with 10 arrays. Hybridization chambers were stuck around each array. *Fem A* and *mec A* sequences from nine *Staphylococci* species were then amplified by a duplex PCR using the consensus primers and products hybridized on the arrays. A negative PCR control was also added. After silver precipitation, the arrays were analyzed in the double CD reader, which was inserted into a computer (Figure 5). These reconstituted data were processed, and the quantification of these 10 arrays obtained after image analysis is shown (Figure 6).

All the detections were specific for their respective products. The consensus *fem A* capture probe and the *mec A* were positive for all the samples, while the specific capture probes only detect their respective products, such as *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, and *S. saprophyticus*. The quantitative data confirmed such conclusions because the signal-to-noise ratio was above 50 for all positive signals.

DISCUSSION

The CD is probably one of the most commonly used data storage support in our daily lives. Here we demonstrated that a CD platform could be adapted for performing DNA analysis while keeping its powerful storage of numeric information. Coupling both genomic and numeric information was achieved through the use of the same physical CD support.

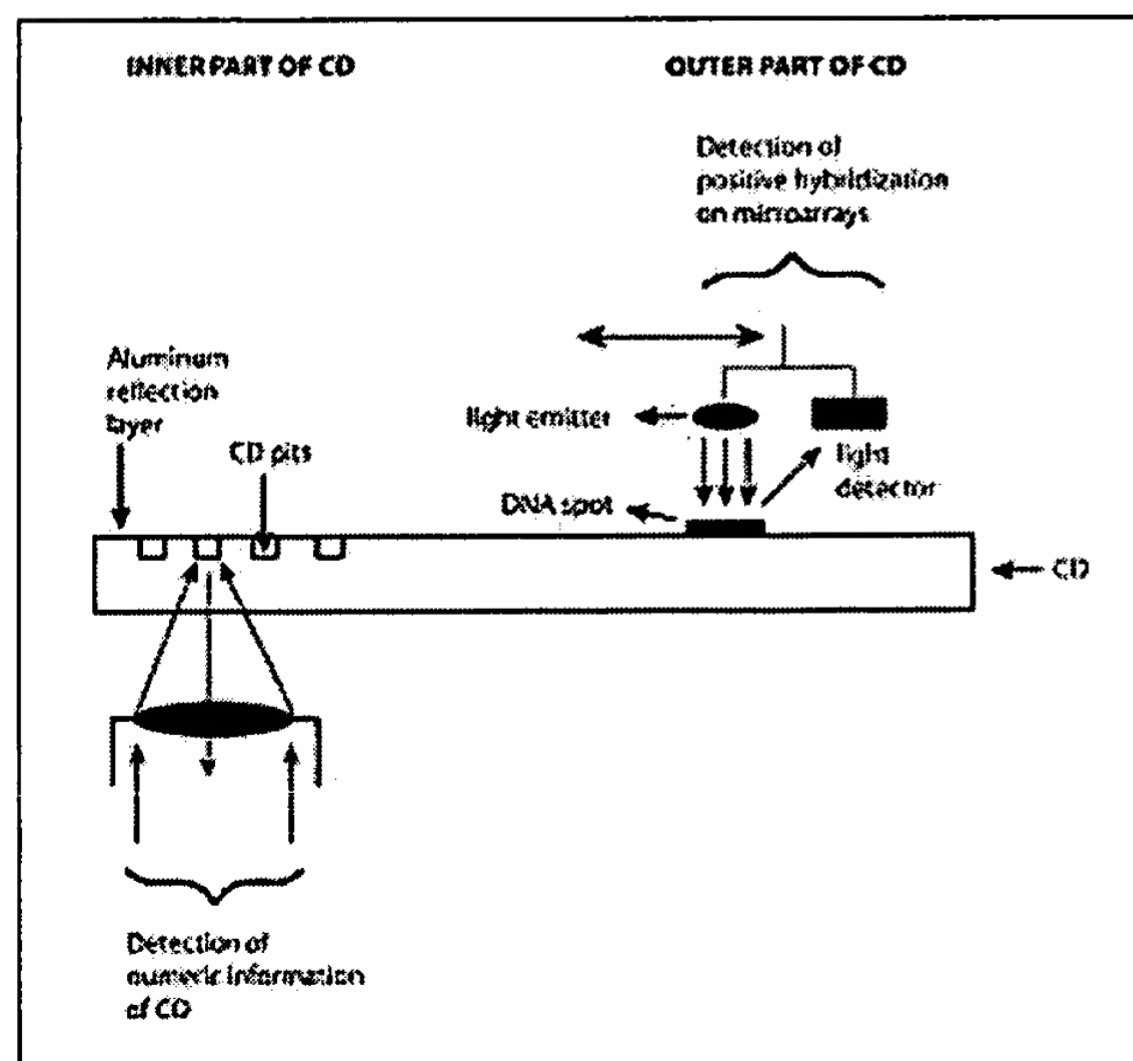


Figure 3. Schematic representation of a reading system for colorimetric CD detection composed of two laser-based devices. The first one is a classic laser CD reader reading the numeric information registered in the inner phase of the CD, and the second one is a photodiode head detecting the laser light diffraction in the presence of positive hybridization onto the microarray of the CD.

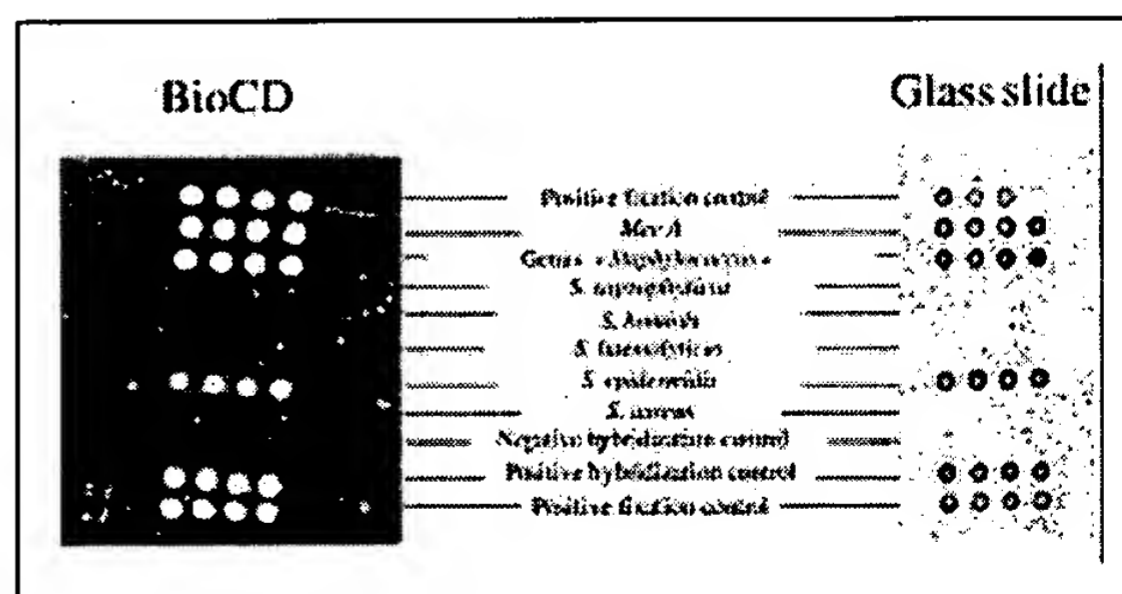


Figure 4. Result of the hybridization of a duplex PCR product made on two plasmids, one containing the *fem A* gene of *S. epidermidis*, and the other one containing the *mec A* gene. The hybridization was performed on a microarray on the surface of a CD and on a glass slide. The array is composed of 11 lines and four columns; each line is composed of four spots of the same DNA capture probes. The sequences detected by the various capture probes are given in the figure. The genus *Staphylococcus* probe recognized the bacteria from *Staphylococcus* genus, and the other five capture probes are specific to five *Staphylococcus* species. Hybridization was performed as described earlier (8). The biotinylated products were incubated with streptavidin-gold conjugate and then with the Silver Blue solution for silver precipitation and colorimetric detection (1).

The inner part of the CD contains all the registered information needed for performing the CD reading and controlling the CD reader. This information also provides the location and identification of the various arrays and the DNA capture probes present on the CD. In addition, it can include the quantification program necessary for the data management. Recordable area is yet another possible alternative for writing and storing the biological results of the microarrays on the CD.

One of the main advantages of the CD is its very large surface, which can afford many different arrays or a few very large ones. Figure 1 shows a CD that has a working surface of 74.5 cm² for biological analysis. Using spots of 0.2 mm every

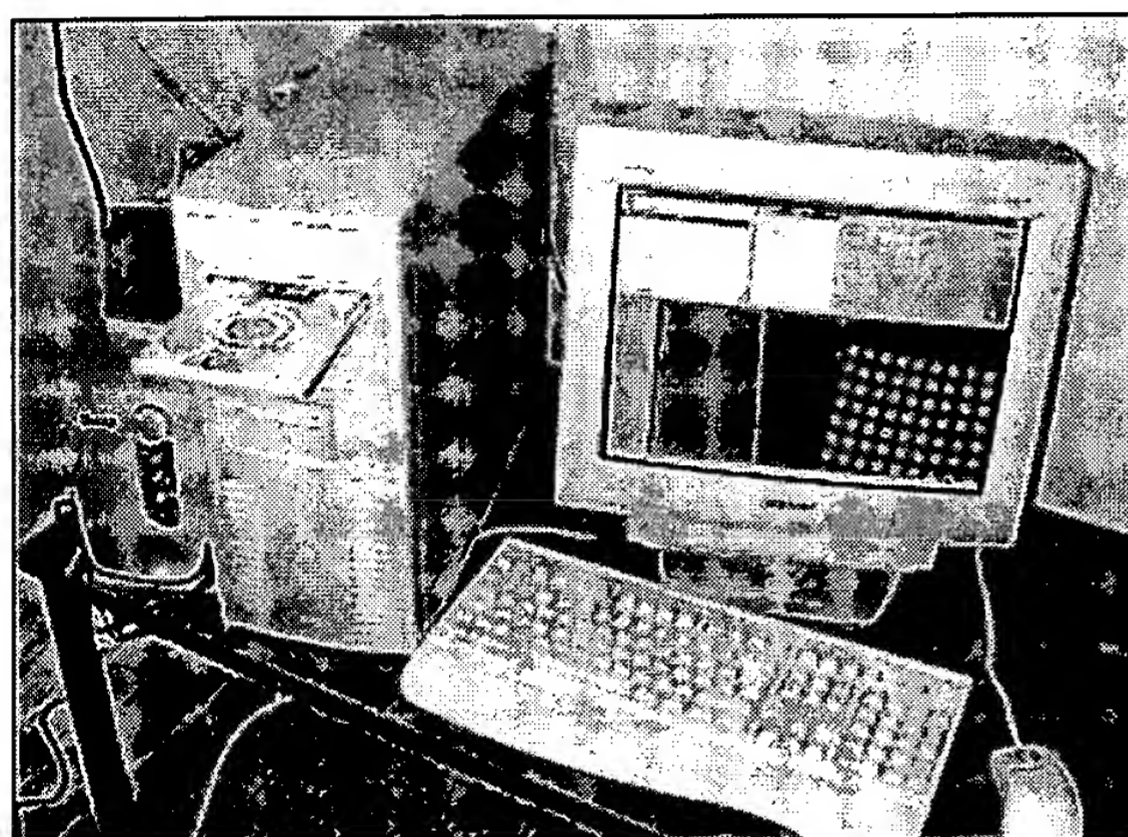


Figure 5. Photograph of the double-sided CD detector. It is composed of a normal laser CD reader to read the numeric information of the CD and a second laser head to detect the presence of positive DNA hybridization onto the microarray of the Bio-CD.

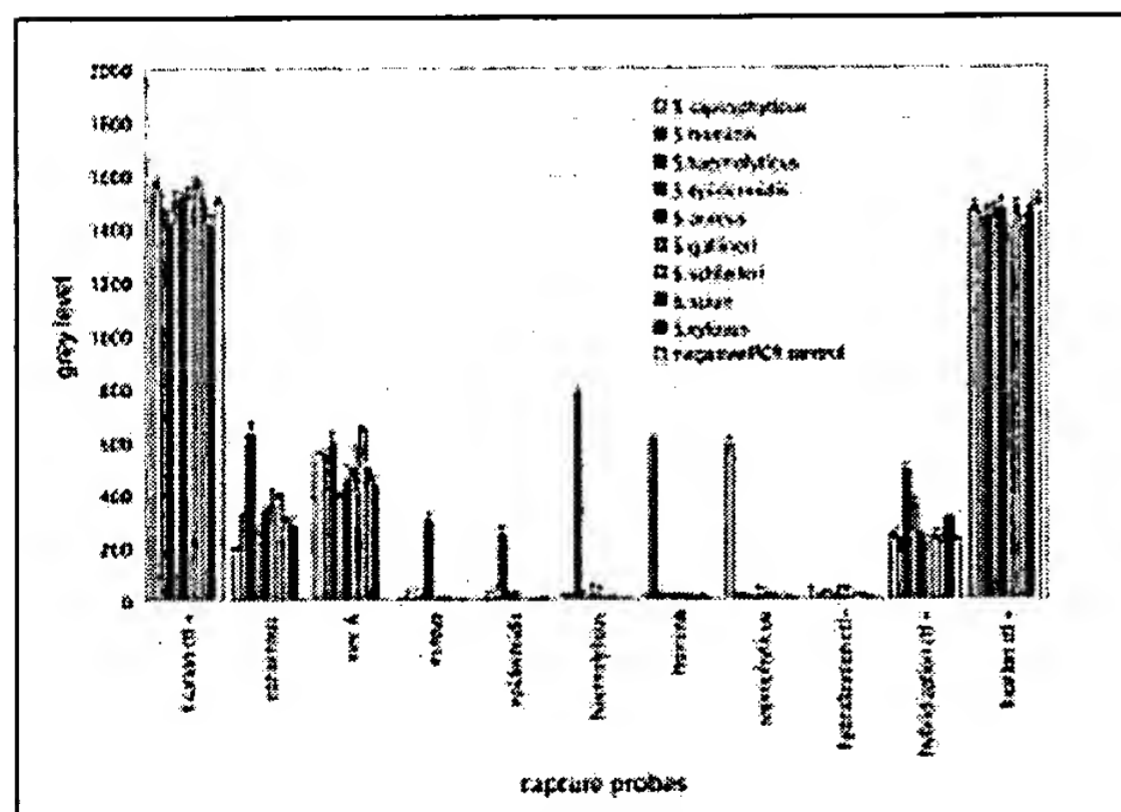


Figure 6. Results of the hybridization of nine duplex PCR products made on nine plasmids containing *fem A* from nine different *Staphylococcus* species in the presence of a second plasmid containing the *mec A* gene. The assay also contains one negative PCR control. The hybridizations were made on 10 microarrays present on the same disc platform. Detection is performed with silver precipitation technology. The nine species are *S. aureus*, *S. epidermidis*, *S. gallineri*, *S. hominis*, *S. saprophyticus*, *S. schleiferi*, *S. sciuri*, *S. simulans*, and *S. xylosus*. Five microliters of each PCR product were first hybridized and washed, incubated with a solution of streptavidin-gold for 45 min, and then washed again and incubated with Silver Blue solution for 10 min (1). The results show the specificity of the detection even between homologous sequences and the simultaneous detection of several samples in one incubation and reading run. The values are the $\bar{x} \pm SD$ of gray level for four spots minus the gray level of the background.

0.4 mm, it would be possible to detect 45 000 sequences on one CD. Both the CD and the CD reader inserted in the computer constitute low-cost technologies.

The fast scanning of the overall CD is another advantage of the system because it can be done within 1 min and data processing is performed automatically by the software, thus making the reading step easy for the user.

In combination with electronic and glass supports, we propose the CD as an alternative platform for making and detecting arrays that are well suited for the routine and multi-sample analysis required in diagnostic DNA and research applications.

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